APRIL, 1925 MEMOIR 90

CORNELL UNIVERSITY

AGRICULTURAL EXPERIMENT STATION

THE PRODUCTION OF VOLATILE FATTY ACIDS IN THE INTESTINAL TRACT OF CALVES FED WHOLE MILK OR CEREAL GRUEL

LEO CHANDLER NORRIS

ITHACA, NEW YORK
PUBLISHED BY THE UNIVERSITY
Received for publication December 16, 1924

3781
C81N
NCLASSED

THE PRODUCTION OF VOLATILE FATTY ACIDS IN THE INTESTINAL TRACT OF CALVES FED WHOLE MILK OR CEREAL GRUEL

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF CORNELL UNIVERSITY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

LEO CHANDLER NORRIS

THE PRODUCTION OF VOLATILE FATTY ACIDS IN THE INTESTINAL TRACT OF CALVES FED WHOLE MILK OR CEREAL GRUEL[1]

LEO CHANDLER NORRIS

In the course of some experiments in rearing dairy calves on cereal gruel instead of whole milk, it became necessary to obtain a clearer conception of what was actually happening within the digestive tract, for observations on calves so fed indicated that there frequently existed a condition of slight indigestion and an abnormal bacterial activity, sufficiently great to slow down the rate of growth.

Because of the large amount of protein in the ration, it was at first thought that such bacterial activity was putrefactive in nature. Experiments (Maynard and Norris, 1922) conducted to show whether or not this was true indicated that no more putrefaction was taking place with the cereal diet than with a milk diet. Bacteriological studies of the effect of carbohydrate feeding (Hull and Rettger, 1917) on the character of the intestinal flora substantiate this conclusion.

These results led to the belief that the bacterial activity obtained was a process of fermentation, and it is the object of this paper to show that such was the case. In order to do this, it became necessary to employ a method which would determine quantitatively the extent of the fermentation taking place when a cereal gruel diet was used in comparison with a milk diet, as some fermentation is probably always present in normal digestion.

METHODS EMPLOYED

A review of the literature of this subject showed that numerous organic acids, including the volatile fatty acids, are produced in the feces of both man and the domestic animals as a result of bacterial activity; also, that these volatile fatty acids are produced by fermentation. The methods used in proving that these acids are the products of fermentation have been well worked out and are applicable to the analysis of organic residues.

The most widely used method and the one employed in this work is that of Duclaux (1865, 1900), who evolved it in identifying, and determining

[1] Also presented to the Faculty of the Graduate School of Cornell University, February, 1924, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENTS. This experiment was conducted in the Department of Animal Husbandry of the New York State College of Agriculture, under the direction of Dr. Leonard A. Maynard, to whom the writer desires to express his appreciation for many suggestions and generous assistance. He desires also to acknowledge his indebtedness to Dr. T. R. Briggs for criticism of the theory upon which the methods used in this problem were based.

the amount of, the fatty acids originating from the alcoholic fermentation of wines. Others have used the same method in determining the fatty acids in cheese (Suzuki, Hastings, and Hart, 1909–10), in corn silage (Dox and Neidig, 1912), and in culture media containing carbohydrates (Seliber, 1910).

Briefly, Duclaux's method is based on the assumption that each of the lower fatty acids, when distilled in very dilute aqueous solution, has its own rate of distillation, whether distilled alone or in the presence of its homologues. This, however, is an approximation, as it is obvious that, unless the solute has the same rate of volatilization as the solvent, the concentration of the solution will change, and with it the rate of distillation of the acid. But it so happens that propionic, isobutyric, and butyric acids form azeotropic mixtures (Lecat, 1918), boiling at the temperatures of 99.98°, 99.3°, and 99.4° C., respectively. These mixtures contain 17.7, 21.0, and 18.4 mol per cent of acid, in the order named. Since the boiling point of each of these azeotropic mixtures is practically 100° C., the rate of volatilization of the acids is very nearly the same as that of water for all solutions up to the concentrations previously mentioned. As a result, their rate of distillation relative to water will not change appreciably during any distillation.

On the other hand, acetic acid does not form an azeotropic mixture, and formic acid forms one with a maximum boiling point of 107.5° C. This mixture contains 22.5 mol per cent of formic acid. Hence, in the absence of mixtures with a constant boiling point very close to 100° C., the rate of distillation of these acids relative to water will change appreciably during the process of distillation, since they do not have the same rate of volatilization as water has. This statement is upheld by Richmond (1895, 1908), who has shown that the rate of distillation of formic and acetic acids rises slightly with increasing concentration, altho to a less extent for the acetic acid. But Richmond has demonstrated also that the change in the rate of distillation of formic and acetic acids, relative to water, during any Duclaux distillation is unimportant if the process is conducted in very dilute solution.

Because of these facts, it is possible to determine without complete separation the quantity of acid present in dilute solution to a fair degree of accuracy. This is true also where more than one acid is present in the solution, provided that the total concentration is sufficiently small so as not to affect the rate of distillation of each. The correctness of this has been shown by Gillespie and Walters (1917), who worked with mixtures of acids of known composition. They found that their actual and their calculated results agreed very closely as long as not more than three acids were present in the solution. When four or more acids were contained in solution, the results were too far in error to be of quantitative value.

The Duclaux method consists of distilling a dilute solution of volatile fatty acids made up to a definite volume of 110 cubic centimeters, collecting the distillate in ten equal fractions of 10 cubic centimeters each, and titrating the acid in each fraction with N/10 barium hydroxide. This process is first applied to solutions of the pure acids and then to unknown mixtures.

Since the rate of distillation of the volatile fatty acids in dilute solution is practically constant, if the distillation is carried out as prescribed above, the quantity of acid distilling in the first 10 cubic centimeters is a definite percentage of the total quantity taken for distillation. This holds also for the first 20 cubic centimeters, the first 30 cubic centimeters, and so forth. Furthermore, this same percentage should theoretically be collected in any given fraction whether or not other acids are distilling at the same time. It was previously pointed out, however, that this is not true in practice, when more than three are present in the distilling mixture in significant quantities. If these data are worked out in advance for the pure acids, it is possible to stop the distillation of an unknown mixture with any fraction and calculate the percentage of any acid in the residue, provided that the qualitative composition of the mixture is known.

Again, if the acid solution is distilled from a volume of 110 cubic centimeters and the distillate is collected in 10-cubic-centimeter fractions, for any one acid, whether alone or, as previously stated, in the presence of not more than two of its homologues, a definite percentage of the quantity of acid distilling in 100 cubic centimeters comes over in the first 10 cubic centimeters, in the first 20 cubic centimeters, in the first 30 cubic centimeters, and so forth. Furthermore, these percentages are not dependent on the concentration of the solution, if it is very dilute, but are constant for each acid.

Gillespie and Walters claim that if the data are calculated as described in the preceding paragraph, the results seem to effect a certain compensation of errors, and in consequence they obtained more accurate results in their experimental work. The first method, however, must be used in determining the total quantity of acid in the distilling flask at the beginning of any Duclaux distillation.

In calculating the results of analysis and in determining the acids present in an unknown mixture, Duclaux used a "cut-and-try" method. This required the making up of an assumed mixture of known volatile acids in such proportions that the percentage of acid distilling in each fraction would approximate the percentage obtained for the corresponding fractions of the unknown mixture. Upson, Plum, and Schott (1917) have pointed out that by using this method of calculation, incorrect conclusions may be drawn from the data. On the other hand, Gillespie and Walters have shown that such errors are not important if a direct method of calculation, such as the algebraic or the graphical one which they describe, is used instead of the "cut-and-try" method.

The application of the method of direct calculation to known mixtures shows that mixtures of two or three acids may be quantitatively analyzed in this manner with reasonable accuracy, but that the error is too great for mixtures of four acids. When four or more acids are present in significant quantities, the solution must be fractionated into mixtures containing only three acids in significant quantities before applying the Duclaux method.

To use the Duclaux method most successfully, the acids met with in the experimental work should be identified qualitatively before calculating the results. Of all the tests proposed, those suggested by Agulhon and rearranged by Dyer (1916–17) seemed the most practicable. These tests depend upon the relative solubility of the iron and copper salts of the fatty acids in various organic solvents.

The Duclaux method depends upon the fact that the lower fatty acids are volatile in steam. Because of this property, almost complete separation of the acids can be made from organic residues contained in water. However, where lactic acid is present in the organic matter, as it may likely be in the case of feces, a modification must be made in the method of steam distillation, as lactic acid is appreciably volatile in steam at 100° C. (Jensen, 1904, and Dox and Neidig, 1912). But when the distillation is carried out under diminished pressure, a separation of the volatile fatty acids can be completely effected (Welde, 1910). Dox and Neidig found that, for all practical purposes, the separation was complete enough when the distillation temperature was maintained between 80° and 85° C.

The Duclaux method used in this work is the modification suggested by Gillespie and Walters, where the results are calculated by the graphical method. Diminished pressure is made use of in separating the fatty acids from the feces, and the acids are identified by the qualitative tests of Agulhon as rearranged by Dyer.

EXPERIMENTAL WORK

In the experimental work, three calves of approximately the same age were used. These calves are designated thruout this paper as A, B, and C, respectively. Calf A received whole milk from birth until the close of the experiment, while B and C were fed whole milk for 35 and 33 days, respectively. Then these last two calves were gradually changed from whole milk to a gruel composed largely of finely ground cereals (Maynard and Norris, 1923). In the case of B the change was completed in 14 days, but 17 days were required for C to make the change, due to a somewhat greater difficulty in handling the cereal gruel. From then until the close of the experiment these calves were restricted to a ration composed entirely of cereal gruel. None of the calves received any hay thruout the period of the experiment, but all were taught to eat a dry mash at an early age, and thereafter were given daily all that they would eat readily.

The experimental period started when the calves were approximately five weeks old, and continued for 28 days. The daily food consumption of each calf for the three days immediately preceding the collection of the feces is given in table 1:

TABLE 1. DAILY FOOD CONSUMPTION FOR THE THREE DAYS IMMEDIATELY PRECEDING COLLECTION OF THE FECES

Calf	Trial no.	Grams of dry matter in			Total grams of food
		Mash	Gruel	Milk	
A	1	301.3	.....	685.8	987.2
	2	251.1	.....	685.8	937.0
	3	150.7	.....	857.3	1,008.0
	4	200.9	.....	857.3	1,058.2
B	1	.....	545.4	304.8	850.2
	2	.....	701.2	171.5	872.6
	3	.....	1,001.6	.....	1,001.6
	4	.....	1,168.6	.....	1,168.6
C	1	124.6	233.7	485.8	844.1
	2	249.1	400.7	342.9	992.7
	3	165.9	801.3	.....	967.3
	4	62.3	801.3	.....	863.6

As far as it could be determined, the length of time it takes for food to pass thru the intestinal tract of the young calf is unknown. Therefore, the food consumption was kept constant for three days preceding the beginning of each collection period. By this means, it was hoped that the feces collected would bear a correct relation to the food consumed daily.

The feces of the calves were collected for the first time when the calves were approximately 40 days old. Four collections were made from each calf at intervals of 7 days. All collections were conducted thru a 24-hour period.

During collections the feces were preserved in covered galvanized pails, previously rinsed with a solution of thymol and ether. Thymol was used, not only because it is a very effective germicide, but also because it is not volatile in steam. The thymol solution was also incorporated into the feces as collected, by thoro stirring. This procedure seems to have effectually stopped bacterial activity.

At the close of the collection period the feces of each calf were weighed, and moisture determinations were made at once. The results are given in table 2:

TABLE 2. FECES EXCRETED DURING 24 HOURS

Calf	Condition of feces	Grams in each trial			
		First trial	Second trial	Third trial	Fourth trial
A	Moist	490.0	720.0	750.0	1,132.0
	Moisture-free	103.0	141.3	136.3	182.8
B	Moist	2,188.0	1,990.0	1,980.0	~~4,062.0~~ 2412.0
	Moisture-free	337.6	306.5	363.7	515.4
C	Moist	1,120.0	1,890.0	2,750.0	~~4,280.0~~ 2700.0
	Moisture-free	196.1	267.4	449.6	431.5

In addition, the reaction of the feces of each calf to litmus was determined and notes were made on color, odor, and mechanical condition.

Duplicate samples of 100 grams were taken of each feces collected except the first. To each of these, 25 cubic centimeters of phosphoric acid was added in order to release any of the volatile fatty acids present in the form of salts. Phosphoric acid was used instead of sulfuric, as the latter tends to break down into sulfites in the presence of organic matter, and the occurrence of these sulfites would make it impossible to separate the volatile fatty acids by steam distillation.

The steam distilling system used was of such a nature that a constant boiling temperature of 80° C., or slightly less, could be maintained. A 100-gram sample of feces was placed in the distilling flask and enough distilled water was added to bring the contents to one and one-half liters. Then the system was exhausted until the water in it would boil at a temperature of 80° C. When the system was heated to this point, the distillation was carried on until the distillate collected had a titratable acidity of three cubic centimeters, or less, of N/10 barium hydroxide per liter. This was not ordinarily attained until about six liters of distillate had passed over. The distillate was then neutralized with N/10 barium hydroxide and distilled into two equal portions in order to separate any alcohols or esters that may have been present in the feces.

Acid production

The residue from the final distillation in the procedure just described, which contained the neutral acids, was then evaporated to a small volume over a water bath, acidified with a slight excess of N/5 sulfuric acid, and was fractionated into three portions in order to assure the presence of not more than three fatty acids in significant quantities. Each frac-

tion was made up to a volume of 110 cubic centimeters, put in a 300-cubic-centimeter round-bottomed flask, and distilled according to the Duclaux method. The results obtained from these distillations were later added together, and they appear in the appendix as a single Duclaux distillation.

In order to prevent condensation at the still head, the distilling flask was completely inclosed in a hollow water bath of copper (Richmond, 1908), the head of which was covered with a copper plug about one and one-half inches deep, packed with asbestos. By this means, it was possible to maintain a constant temperature at the still head of approximately 99° C.

The distillate was collected in successive 10-cubic-centimeter portions, in small graduated cylinders, and was titrated with N/10 barium hydroxide. The titration figures so obtained constitute the basis for calculating the results given in this paper.

Before these calculations could be made, it was necessary to have the percentage constants for the pure acids. As the writer thought it best to use constants derived by his own procedure, rather than those of Duclaux or others, three Duclaux distillations were made on the lower fatty acids found by qualitative test to be present in the feces. These are given in tables 3, 4, 5, and 6:

TABLE 3. AMOUNT OF ACETIC ACID COLLECTED, EXPRESSED AS PERCENTAGE OF THE QUANTITY IN 100 CUBIC CENTIMETERS OF DISTILLATE

Volume of distillate (cubic centimeters)	Per cent of acetic acid in each trial			Average per cent for all trials
	First trial	Second trial	Third trial	
10	7.82	7.82	7.82	7.83
20	15.98	16.07	16.11	16.05
30	24.59	24.66	24.63	24.63
40	33.41	33.48	33.40	33.43
50	42.56	42.63	42.57	42.59
60	52.26	52.35	52.37	52.33
70	62.40	62.51	62.51	62.47
80	73.53	73.67	73.62	73.61
90	85.77	85.83	85.90	85.83
100	100.00	100.00	100.00	100.00

TABLE 4. AMOUNT OF ACETIC ACID COLLECTED, EXPRESSED AS PERCENTAGE OF THE TOTAL QUANTITY TAKEN FOR DISTILLATION

Volume of distillate (cubic centimeters)	Per cent of acetic acid in each trial			Average per cent for all trials
	First trial	Second trial	Third trial	
10	6.33	6.30	6.31	6.31
20	12.93	12.96	12.93	12.94
30	19.89	19.89	19.76	19.85
40	27.03	27.00	26.80	26.94
50	34.43	34.38	34.15	34.32
60	42.28	42.21	42.02	42.17
70	50.49	50.41	50.16	50.35
80	59.50	59.41	59.07	59.33
90	69.40	69.22	68.93	69.18
100	80.90	80.65	80.24	80.60

TABLE 5. AMOUNT OF PROPIONIC ACID COLLECTED, EXPRESSED AS PERCENTAGE OF THE QUANTITY IN 100 CUBIC CENTIMETERS OF DISTILLATE

Volume of distillate (cubic centimeters)	Per cent of acetic acid in each trial			Average per cent for all trials
	First trial	Second trial	Third trial	
10	12.16	12.25	12.19	12.20
20	23.81	23.94	23.64	23.80
30	35.01	35.94	34.89	35.01
40	45.69	45.87	45.72	45.76
50	56.05	56.16	56.04	56.08
60	65.84	65.95	65.83	65.87
70	75.16	75.30	75.21	75.22
80	84.05	84.02	84.07	84.05
90	92.35	92.36	92.30	92.34
100	100.00	100.00	100.00	100.00

TABLE 6. AMOUNT OF PROPIONIC ACID COLLECTED, EXPRESSED AS PERCENTAGE OF THE TOTAL QUANTITY TAKEN FOR DISTILLATION

Volume of distillate (cubic centimeters)	Per cent of acetic acid in each trial			Average per cent for all trials
	First trial	Second trial	Third trial	
10	11.28	11.32	11.28	11.29
20	22.09	22.11	21.89	22.03
30	32.48	32.44	32.30	32.41
40	42.39	42.36	42.33	42.36
50	52.00	51.87	51.88	51.92
60	61.07	60.91	60.95	60.98
70	69.73	69.54	69.62	69.63
80	77.97	77.60	77.83	77.80
90	85.67	85.30	85.44	85.47
100	92.78	92.36	92.57	92.57

Two sets of constants are given in these tables, because both are necessary in calculating the results of unknown mixtures. That they do not vary widely from those obtained by other experimenters doing similar work is shown by the comparative results given in tables 7, 8, 9, and 10:

TABLE 7. RESULTS OF DIFFERENT WORKERS FOR ACETIC ACID, EXPRESSED AS PERCENTAGE OF THE QUANTITY IN 100 CUBIC CENTIMETERS OF DISTILLATE

Volume of distillate (cubic centimeters)	Per cent of acetic acid in 100 cubic centimeters of distillate				
	Duclaux	Schott	Plum	Gillespie and Walters	Norris
10	7.4	7.82	7.30	8.0	7.83
20	15.2	16.0	15.17	16.3	16.05
30	23.4	24.4	23.55	24.6	24.63
40	32.0	33.2	32.00	33.5	33.43
50	40.9	42.4	41.07	42.7	42.59
60	50.5	52.1	50.72	52.1	52.33
70	60.6	62.4	61.02	62.4	62.47
80	71.9	73.5	72.26	73.4	73.61
90	84.4	85.8	84.96	85.7	85.83
100	100.0	100.0	100.00	100.0	100.00

TABLE 8. RESULTS OF DIFFERENT WORKERS FOR ACETIC ACID, EXPRESSED AS PERCENTAGE OF THE TOTAL QUANTITY TAKEN FOR DISTILLATION

Volume of distillate (cubic centimeters)	Per cent of acetic acid in total quantity				
	Duclaux	Richmond	Schott	Gillespie and Walters	Norris
10	5.9	5.9	6.33	6.4	6.31
20	12.2	12.2	12.97	13.0	12.94
30	18.7	18.8	19.80	19.7	19.85
40	25.6	25.6	26.94	26.7	26.94
50	32.7	32.7	34.32	34.1	34.32
60	40.4	40.5	42.20	41.6	42.17
70	48.7	48.7	50.53	49.9	50.35
80	57.5	57.9	59.34	58.7	59.33
90	67.5	67.7	69.34	68.5	69.18
100	80.0	79.8	80.98	79.9	80.60

TABLE 9. RESULTS OF DIFFERENT WORKERS FOR PROPIONIC ACID, EXPRESSED AS PERCENTAGE OF THE QUANTITY IN 100 CUBIC CENTIMETERS OF DISTILLATE

Volume of distillate (cubic centimeters)	Per cent of propionic acid in 100 cubic centimeters of distillate				
	Duclaux	Schott	Plum	Gillespie and Walters	Norris
10	12.1	11.9	12.16	12.0	12.20
20	24.0	23.3	23.75	23.7	23.80
30	35.3	34.4	34.85	34.9	35.01
40	46.2	45.3	45.66	45.8	45.76
50	56.8	55.7	56.14	56.3	56.08
60	66.7	65.8	66.12	66.2	65.87
70	76.2	75.3	75.65	75.7	75.22
80	85.0	84.3	84.63	84.5	84.05
90	93.0	92.4	92.68	92.6	92.34
100	100.0	100.0	100.00	100.0	100.00

Gillespie and Walters made use of $7\frac{1}{2}\times10$-inch coordinate paper with 10 lines to the half inch, in calculating by the graphical method the results of a Duclaux analysis where only two acids were present in significant quantities. L_{10}, L_{20}, and so forth, are taken as the distillation constants of the less volatile acid, and M_{10}, M_{20}, and so forth, as the distilla-

TABLE 10. RESULTS OF DIFFERENT WORKERS FOR PROPIONIC ACID, EXPRESSED AS PERCENTAGE OF THE TOTAL QUANTITY TAKEN FOR DISTILLATION

Volume of distillate (cubic centimeters)	Per cent of propionic acid in total quantity				
	Duclaux	Richmond	Schott	Gillespie and Walters	Norris
10	11.5	10.5	11.26	11.2	11.29
20	22.8	21.1	22.12	22.2	22.03
30	33.5	31.5	32.61	32.7	32.41
40	44.0	41.4	42.96	42.9	42.36
50	54.0	51.3	52.76	52.7	51.92
60	63.3	60.6	62.33	62.0	60.98
70	72.5	69.5	71.28	70.9	69.63
80	81.0	78.0	79.84	79.1	77.80
90	88.5	85.8	87.58	86.7	85.47
100	95.0	92.8	94.74	93.6	92.57

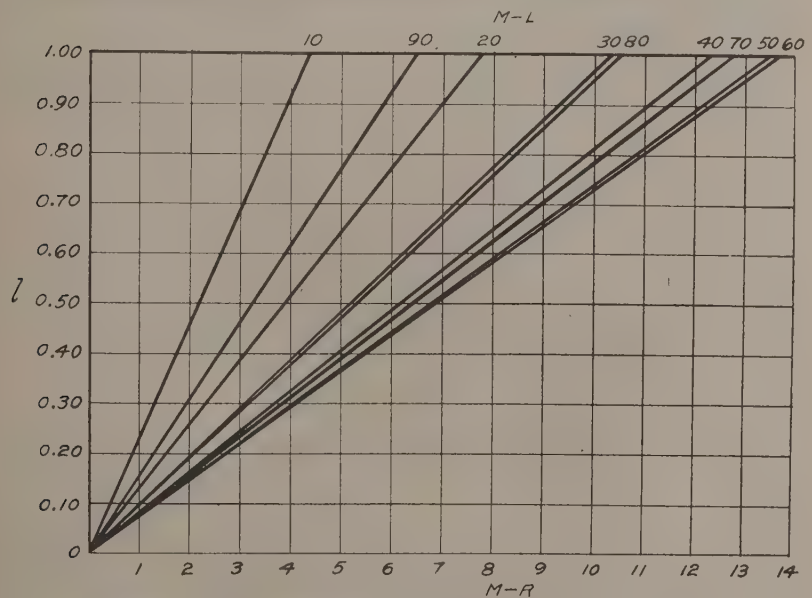

FIGURE 1. RELATION BETWEEN l AND M_{10}-R_{10}, M_{20}-R_{20}, ... , M_{90}-R_{90}

tion constants of the more volatile acid (tables 3 and 5). "Less volatile" and "more volatile" refer to volatility from aqueous solution, not that of the pure acids. The fraction of less volatile acid in 100 cubic centimeters of distillate is taken as l. This is the unknown quantity in the calculations and is taken from the vertical axis in the graph for acetic and propionic acids (figure 1.) R is taken as the percentage constant of the unknown mixture. On the horizontal axis, where l equals 1.00, the values of $M_{10} - L_{10}$, $M_{20} - L_{20}$, ... , $M_{90} - L_{90}$ are marked off to the right. A line is then drawn from the point of origin thru each of these points. This gives nine lines representing the relation between l and $M_{10} - R_{10}$, $M_{20} - R_{20}$, and so forth, respectively. For every analysis the differences, $M_{10} - R_{10}$, and so forth, must be found and marked off along the horizontal axis on the corresponding line of the graph. The values of l are then read off on the l axis at the left. This gives nine values for l, the average of which represents the fraction of L, the less volatile acid, in 100 cubic centimeters of distillate. Then $1.00 - l$ will give the fraction of the more volatile acid, M, in 100 cubic centimeters of distillate. These values, when divided by their respective fractions of pure acid appearing in 100 cubic centimeters of distillate (tables 4 and 6), will give the acid originally present in 110 cubic centimeters of distillate.

From these results the total amount of each acid excreted during a period of 24 hours can be readily computed. The results of the Duclaux analysis of the acids obtained from the feces appear in table 15, in the appendix, and the total acid excreted daily is shown in table 11:

TABLE 11. AMOUNT OF ACID EXCRETED IN 24 HOURS

Trial	Calf	Acids present	Per cent of each acid	Cubic centimeters of N/10 acid per 100 cubic centimeters of solution	Cubic centimeters of N/10 acid per 110 cubic centimeters of solution	Total acid excreted daily (cubic centimeters)
First	A	Propionic	48.47	45.43	49.06	240.39
		Acetic	51.53	48.30	59.93	293.66
		Total	.....	.....	.....	534.05
	B	Propionic	44.82	61.86	66.80	1,461.58
		Acetic	56.18	76.16	94.49	2,067.44
		Total	.....	.....	.....	3,529.02
	C	Propionic	52.42	75.19	81.19	909.33
		Acetic	47.58	68.25	84.68	948.32
		Total	.....	.....	.....	1,857.65

TABLE 11 (*concluded*)

Trial	Calf	Acids present	Per cent of each acid	Cubic centimeters of N/10 acid per 100 cubic centimeters of solution	Cubic centimeters of N/10 acid per 110 cubic centimeters of solution	Total acid excreted daily (cubic centimeters)
Second	A	Propionic	34.06	44.50	48.05	345.96
		Acetic	65.94	86.16	106.90	769.68
		Total	.....	.....	.....	1,115.64
	B	Propionic	47.99	79.64	86.00	1,711.40
		Acetic	52.01	86.31	107.08	2,130.89
		Total	.....	.....	.....	3,842.29
	C	Propionic	44.33	60.64	65.48	1,237.57
		Acetic	55.67	76.16	94.49	1,785.86
		Total	.....	.....	.....	3,023.43
Third	A	Propionic	35.54	35.75	38.60	289.50
		Acetic	64.46	64.85	80.46	603.45
		Total	.....	.....	.....	892.95
	B	Propionic	38.02	32.02	34.57	684.49
		Acetic	61.98	52.19	64.75	1,282.05
		Total	.....	.....	.....	1,966.54
	C	Propionic	55.86	102.42	110.59	3,041.23
		Acetic	44.14	80.93	100.41	2,761.28
		Total	.....	.....	.....	5,802.51
Fourth	A	Propionic	30.12	40.36	43.47	492.08
		Acetic	69.88	93.40	115.88	1,311.76
		Total	.....	.....	.....	1,803.84
	B	Propionic	50.82	67.85	73.26	1,766.03
		Acetic	40.18	65.67	81.48	1,965.30
		Total	.....	.....	.....	3,731.33
	C	Propionic	54.17	92.54	99.92	2,697.84
		Acetic	45.83	78.30	97.15	2,623.05
		Total	.....	.....	.....	5,320 89

The results of making the Dyer qualitative tests on the neutral residues from the Duclaux distillation revealed the presence of propionic and acetic acids. These tests were always very distinct and were made previous to the calculation of the Duclaux analysis. No other acids were present in sufficient quantities to be revealed by these tests, altho at times there was a slight butyric odor given off during the Duclaux distillation. The distillation constants of the acid mixtures also indicated the presence of acetic and propionic acids, for these constants were always intermediate in respect to those for pure acetic and pure propionic acids. This fact alone would not necessarily prove the presence of these acids, but combined with the Dyer qualitative tests it is believed to be sufficient evidence of their presence in the feces of growing calves.

Gillespie and Walters showed that, where the values of l, the fraction of the less volatile acid, show a persistent upward trend, either the wrong acids have been assumed or one or more acids have been neglected. In most instances, the writer found that his values for l showed a significant, altho not large, upward trend. This seems to indicate the presence of very small quantities of one more acid, probably butyric. By recalculating to find this acid with acetic and propionic acids, values for l might be obtained which would show no significant trend in either direction; but as even this would not definitely prove the existence of butyric acid in the feces of calves without evidence to that effect by means of the qualitative test, such recalculations were not made.

It is of interest here to compare the results of this work with those which Bosworth and his coworkers (1922) obtained from an analysis of the acid stools of breast-fed and bottle-fed infants. These writers found formic and acetic acids in the stools of the breast-fed infants, and acetic acid in those of the bottle-fed infants. There were also strong indications of the presence of small amounts of higher volatile fatty acids in the stools of the bottle-fed infants, but no identification of them was made.

Alcohol production

The ester-alcohol distillate obtained by fractionating the neutral acid distillate of the feces was repeatedly divided by distillation into two equal portions until the final distillate amounted to about 50 cubic centimeters. This was saponified for three hours, at the temperature of boiling water, by means of 20 cubic centimeters of a 20-per-cent solution of potassium hydroxide. It was then cooled, diluted with distilled water, and distilled to drive off any alcohols, either free or in ester combination. The residue was acidified with N/5 sulfuric acid and distilled with repeated additions of water. The distillate from this was neutralized with N/10 barium hydroxide.

The highest acidity of this distillate in six different cases was slightly less than 4 cubic centimeters of N/10 acid, indicating that acids in ester

combination were present in very insignificant quantities, if at all. No Duclaux determinations on this acid were attempted, nor was any further effort made to determine acids in ester combination.

The solution of alcohols obtained by saponification was oxidized in a tightly stoppered pressure bottle with 50 cubic centimeters of potassium bichromate solution, consisting of 10 grams of potassium bichromate and 20 grams of concentrated sulfuric acid made up to 100 cubic centimeters. The oxidation process was carried on over a water bath and was considered complete at the end of from four to five hours. Any excess chromic acid was removed by means of zinc in the presence of sulfuric acid. The volatile fatty acids were then distilled by repeated additions of water, neutralized with N/10 barium hydroxide, and evaporated to a small volume. The barium was removed with N/5 sulfuric acid, and the fatty acids were distilled by the Duclaux method. The procedure followed in making these determinations was essentially the same as that used by Suzuki, Hastings, and Hart (1909–10) in ascertaining the production of alcohols and esters in cheddar cheese.

Significant quantities of acid were obtained by the oxidation of alcohols in all but two instances. No explanation can be given for the low results in these two cases.

On applying the Dyer qualitative test to the residues of the Duclaux distillations of the acid mixtures obtained by the oxidation of the alcohols, the presence of acetic acid was revealed in every trial. This showed that considerable ethyl alcohol was present in the feces. But in only two cases were distinct tests for propionic acid given. The Duclaux distillation constants also indicated the presence of only very small quantities of propionic acid, or none at all. No other acids could be identified qualitatively.

The results of the Duclaux distillations of the acids obtained by the oxidation of the alcohols of the feces are given in table 16, in the appendix. The amount of acid obtained by the oxidation of alcohol excreted in 24 hours is shown in table 12:

TABLE 12. AMOUNT OF ACID OBTAINED BY THE OXIDATION OF ALCOHOL EXCRETED IN 24 HOURS

Trial	Calf	Acids present	Per cent of each acid	Cubic centimeters of N/10 acid per 100 cubic centimeters of solution	Cubic centimeters of N/10 acid per 110 cubic centimeters of solution	Total acid excreted daily (cubic centimeters)
First	A	Propionic	None	.....	.....	.....
		Acetic	100.00	91.00	116.63	571.49
		Total	.....	.....	.....	571.49
	B	Propionic	None	.....	.....	.....
		Acetic	100.00	23.32	28.93	632.99
		Total	.....	.....	.....	632.99
	C	Propionic	None	.....	.....	.....
		Acetic	100.00	28.86	35.78	399.50
		Total	.....	.....	.....	399.50
Second	A	Propionic	None	.....	.....	.....
		Acetic	100.00	160.75	199.44	1,435.97
		Total	.....	.....	.....	1,435.97
	B	Propionic	None	.....	.....	.....
		Acetic	100.00	130.82	162.31	3,229.97
		Total	.....	.....	.....	3,229.97
	C	Propionic	None	.....	.....	.....
		Acetic	100.00	121.45	150.68	2,847.85
		Total	.....	.....	.....	2,847.85
Third	A	Propionic	7.84	7.12	7.69	57.68
		Acetic	92.16	83.71	103.86	778.95
		Total	.....	.....	.....	836.63
	B	Propionic	None	.....	.....	.....
		Acetic	100.00	166.58	206.67	4,092.07
		Total	.....	.....	.....	4,092.07
	C	Propionic	3.84*	6.44	6.95	191.12
		Acetic	96.16	167.61	207.95	5,718.63
		Total	.....	.....	.....	5,909.75

* This propionic acid was not identified by the qualitative test.

TABLE 12 (*concluded*)

Trial	Calf	Acids present	Per cent of each acid	Cubic centimeters of N/10 acid per 100 cubic centimeters of solution	Cubic centimeters of N/10 acid per 110 cubic centimeters of solution	Total acid excreted daily (cubic centimeters
Fourth	A	Propionic	13.68	15.81	17.07	193.23
		Acetic	86.32	99.73	123.73	1,400.62
		Total	.....	.....	.....	1,593.85
	B	Propionic	None	.....	.....	.....
		Acetic	100.00	89.14	110.60	2,667.67
		Total	.....	.....	.....	2,667.67
	C	Propionic	None	.....	.....	.....
		Acetic	100.00	189.03	234.55	6,332.85
		Total	.....	.....	.....	6,332.85

The presence of so much acid and alcohol in the feces of calves may at first seem unreasonable, but in view of the fact that Bosworth and his coworkers (1922) found as much as 151.3 cubic centimeters of N/10 volatile fatty acid in the acid stools of bottle-fed infants, this is not so remarkable.

CONCLUSIONS

The results of this experiment indicate a much greater production of volatile fatty acids and of alcohols with a cereal-gruel diet, rich in starch, than with a whole-milk diet. That this is caused by fermentation thru the activity of bacteria cannot be definitely stated. However, Harden (1901) has shown that *B. coli communis*, isolated from the feces of both man and animals, will ferment glucose to acetic acid, alcohol, and other products. It is known also that propionic acid is produced thru the activity of certain bacteria, and that it is found in the feces of animals. Therefore it seems reasonable to conclude that the presence of the volatile fatty acids and of alcohols in the feces of calves is caused by fermentation. The comparative results on which these conclusions are based are presented in table 13.

It is seen from this table that the calves which received cereal gruel excreted daily from three to four times as much acid and alcohol, on the average, as did the calf which received whole milk. This is true also when the results are figured on the basis of acid and alcohol excreted per gram of dry matter eaten daily. These results were obtained in spite of the

fact that calves B and C received some whole milk, but in decreasing amounts, during the first seven and ten days, respectively, of the experimental period, at which time the calves were in the later stage of the change from milk to gruel.

TABLE 13. COMPARISON OF THE DAILY EXCRETION OF FATTY ACIDS AND OF ALCOHOL, FROM A WHOLE-MILK DIET AND FROM A CEREAL-GRUEL DIET

Calf	Trial	Diet	N/10 acid excreted daily (cubic centimeters)	N/10 acid from alcohol excreted daily (cubic centimeters)	N/10 acid per gram of dry matter eaten (cubic centimeters)	N/10 acid from alcohol per gram of dry matter eaten (cubic centimeters)	Per cent of water in feces
A	First...	Milk.....	534.05	571.49	0.58	0.58	78.98
	Second.	Milk.....	1,115.64	1,435.97	1.19	1.53	80.37
	Third..	Milk.....	892.95	8 6.63	0.89	0.83	81.83
	Fourth.	Milk.....	1,803.84	1,593.85	1.70	1.51	83.85
	Average	.....	1,086.62	1,109.49	1.09	1.11	81.26
B	First...	Milk and gruel	3,529.02	632.99	4.15	0.74	84.56
	Second.	Milk and gruel	3,842.29	3,229.97	4.40	3.70	84.60
	Third..	Gruel.....	1,966.54	4,092.07	1.96	4.09	81.63
	Fourth.	Gruel.....	3,731.33	2,667.67	3.19	2.28	78.63
	Average	.....	3,267.30	2,655.68	3.43	2.70	82.35
C	First...	Milk and gruel	1,857.65	399.50	2.20	0.47	82.49
	Second.	Milk and gruel	3,023.43	2,847.85	3.05	2.87	85.85
	Third..	Gruel.....	5,802.75	5,909.75	6.00	6.11	83.65
	Fourth.	Gruel.....	5,320.89	6,332.85	6.16	7.33	84.02
	Average	.....	4,001.12	3,872.49	4.35	4.20	84.00

At first thought, this variation in acid and alcohol excretion between the different diets might be explained on the basis of the relative rate of progress of the feces thru the lower intestinal tract, if it is assumed that there would be greater absorption of acid and alcohol with a condition of constipation than with a condition of diarrhea. However, none of the calves suffered from constipation at any time during the experiment, and the consistency of their feces, as measured by water content, was in every case very nearly the same. It does not seem, therefore, that the rate of progress of the feces thru the lower intestinal

tract could have been widely different. Furthermore, in those instances in which the percentage of water in the feces is practically the same, a much greater elimination of acid and alcohol occurs with the cereal-gruel diet than with the whole-milk diet.

It was observed that the feces of the calf which received whole milk were always non-acid to litmus, while the feces of the other calves were constantly acid to litmus. In addition, the former were uniformly medium to light in color, while the latter were always very dark. The feces of calves B and C were consistently slimy or stringy, and bits of undigested meal were present, but no distinctly bad odor was evident with any collection.

These conditions, together with a greater elimination of acid and alcohol, were correlated with an increased excretion of dry matter and a decreased digestibility. This is seen from the data presented in table 14, in which the dry matter eaten daily, the dry matter excreted daily, and the percentage digestibility of the food intake, are shown:

TABLE 14. DRY MATTER EATEN AND EXCRETED DAILY, AND PERCENTAGE DIGESTIBILITY OF DRY MATTER EATEN

Calf	Trial	Age (days)	Per cent increase in weight for preceding week	Grams of dry matter eaten daily	Grams of dry matter excreted daily	Per cent of dry matter digested
A	First	39	6	987.17	103.00	87.57
	Second	46	11	936.95	141.34	84.92
	Third	53	12	1,007.96	136.28	86.48
	Fourth	60	12	1,058.19	182.82	83.68
	Average	49.5	10.25	997.57	140.86	85.66
B	First	43	5	850.15	337.63	60.30
	Second	50	5	872.15	306.46	64.88
	Third	57	0	1,001.64	363.73	63.69
	Fourth	64	3	1,168.58	515.44	55.90
	Average	53.5	3.25	973.25	380.82	61.19
C	First	41	12	844.07	196.11	76.77
	Second	48	11	992.68	267.44	73.04
	Third	55	8	967.25	449.63	53.52
	Fourth	62	0	863.59	431.46	50.06
	Average	51.5	7.75	916.90	336.16	63.35

The figures for percentage digestibility are not true digestibility coefficients, as the daily excretion of dry matter is not an average for a given period of time. On the other hand, the food intake was constant for three days preceding the collection of the feces. Notwithstanding this, the data are too significant to be neglected, since a lower percentage digestibility was constantly obtained where the diet was mainly cereal gruel.

The correlation of the rise in acid and alcohol production with an increase in excretion of dry matter and a decrease in digestibility seems to indicate that calves of the ages given in table 14 cannot completely digest rations which are relatively rich in starch. This is in agreement with the work of Shaw, Woodward, and Norton (1912) on younger calves.

The greater production of volatile fatty acids and of alcohols is presumably, then, a result of this inability to digest starch well, in consequence of which the lower intestinal tract becomes relatively rich in undigested and unresorbed carbohydrate residues, a medium favorable to the development of aciduric bacteria.

SUMMARY

A method for the identification and the quantitative determination of the volatile fatty acids and alcohols in the feces of domestic animals is presented herein.

Acetic and propionic acids were found in the feces of growing calves. These acids were present in an approximate 1:1 ratio. No other acids of the fatty acid series were revealed by qualitative test, altho there were slight indications of the presence of butyric acid.

Ethyl alcohol was present in large quantities in the feces, but propyl was identified in only two instances and then in only very small amounts. The presence of esters was not proved.

The calves which received a cereal-gruel diet excreted daily a much larger quantity of acid and alcohol than the one which received a whole-milk diet.

The presence of such large quantities of acid and alcohol in the feces of calves fed a cereal-gruel diet indicates that young calves do not have the power to completely digest large amounts of carbohydrates consisting essentially of starches. Therefore the partially digested food residues are acted upon by bacteria, resulting in the production of acids and alcohols.

REFERENCES CITED

BOSWORTH, ALFRED W., AND OTHERS. Studies of infant feeding. XVII. A bacteriochemical study of the acid stools excreted by breast-fed and bottle-fed infants. Amer. journ. diseases of children **23**:323–337. 1922.

DOX, ARTHUR W., AND NEIDIG, RAY E. The volatile aliphatic acids of corn silage. Iowa Agr. Exp. Sta. Research bul. 7:1–32. 1912.

DUCLAUX, E. De l'origine de l'acide acétique produit pendant la fermentation alcoolique. *In* Études relatives à l'absorption de l'ammoniaque et à la production d'acides gras volatils pendant la fermentation alcoolique. École Normale Supérieure. Ann. sci. **2**:270–290. 1865.

———————— Fermentation alcoolique. *In* Traité de microbiologie **3**:1–741. (Reference on p. 384.) 1900.

DYER, D. C. A new method of steam distillation for the determination of the volatile fatty acids, including a series of colorimetric qualitative reactions for their identification. Journ. biol. chem. **28**:445–473. 1916–17.

GILLESPIE, L. J., AND WALTERS, E. H. The possibilities and limitations of the Duclaux method for the estimation of volatile acids. Amer. Chem. Soc. Journ. **39**[2]:2027–2055. 1917.

HARDEN, ARTHUR. The chemical action of *Bacillus coli communis* and similar organisms on carbohydrates and allied compounds. Chem. Soc. [London]. Journ., Trans. **79**:610–628. 1901.

HULL, THOMAS G., AND RETTGER, LEO. The influence of milk and carbohydrate feeding on the character of intestinal flora. IV. Diet versus bacterial implantation. Journ. bact. **2**:47–69. 1917.

JENSEN, ORLA. Biologische Studien über den Käsereifungsprozess unter spezieller Berücksichtigung der flüchtigen Fettsäuren. Landw. Jahrb. Schweiz **18**:314–400. (Reference on p. 315.) 1904.

LECAT, M. La tension de vapeur des mélanges de liquides, l'azeotropisms, p. 1–319. 1918.

MAYNARD, L. A., AND NORRIS, L. C. The excretion of indican and phenols by calves: an abstract. Amer. Soc. Anim. Prod. Proc. **1921**:75–76. 1922.

————————. A system of rearing calves with limited use of milk. Journ. dairy sci. **6**:483–499. 1923.

RICHMOND, H. DROOP. Duclaux's method for the estimation of "volatile fatty acids," and the laws governing "volatility" deduced therefrom, and their application to analysis, more especially to that of butter. I. The mathematical deductions from the distillation of acids of the Cn $H_{2n}+1$ COOH series. Analyst **20**:193–198. 1895.

———————— Studies in steam distillation. III. Formic and acetic acids. Analyst **33**:305–313. 1908.

SELIBER, G. Détermination des acides volatils dans les produits de fermentation de quelques microbes d'après la méthode de Duclaux. Acad. sci. [Paris]. Compt. rend. **150**:1267–1270. 1910.

SHAW, R. H., WOODWARD, T. E., AND NORTON, R. P. Digestion of starch by the young calf. Journ. agr. res. **12**:575–578. 1912.

SUZUKI, S. K., HASTINGS, E. G., AND HART, E. B. The production of volatile fatty acids and esters in cheddar cheese and their relation to the development of flavor. Journ. biol. chem. **7**:431–458. 1909–10.

UPSON, FRED W., PLUM, H. M., AND SCHOTT, J. E. On the Duclaux method for the estimation of the volatile fatty acids. Amer. Chem. Soc. Journ. **39**:731–742. 1917.

WELDE, ERNST. Eine neue Methode zur quantitativen Bestimmung flüchtiger Fettsäuren. Biochem. Ztschr. **28**:504–522. 1910.

APPENDIX

TABLE 15. RESULTS OF THE DUCLAUX DISTILLATION OF ACIDS OBTAINED FROM FECES OF YOUNG CALVES

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M-R$	l
First	A	10	9.50	9.50	10.13	2.07	0.475
		20	9.78	19.27	20.56	3.24	0.420
		30	8.93	28.20	30.08	4.93	0.477
		40	8.97	37.16	39.65	6.11	0.510
		50	8.93	46.09	49.17	6.91	0.532
		60	8.89	54.93	58.65	7.22	0.555
		70	8.93	63.90	68.17	7.05	0.568
		80	9.32	73.22	78.11	5.95	0.585
		90	9.74	82.96	88.50	3.84	0.586
		100	10.77	93.73	100.00	.....	.....
		Average	.....	.....	.....	.....	0.5153
	B	10	13.36	13.36	9.68	2.52	0.575
		20	13.78	27.14	19.66	4.14	0.535
		30	13.57	40.72	24.49	5.52	0.535
		40	13.22	53.92	39.06	6.70	0.547
		50	13.32	67.24	48.71	7.37	0.550
		60	13.29	80.53	58.34	7.53	0.555
		70	13.57	94.10	68.17	7.05	0.554
		80	13.89	107.98	78.23	5.83	0.556
		90	14.42	122.40	88.68	3.65	0.556
		100	15.62	138.02	100.00	.....	.....
		Average	.....	.....	.....	.....	0.5518
	C	10	14.85	14.85	10.35	1.85	0.422
		20	14.46	29.31	20.43	3.37	0.437
		30	14.12	43.43	30.28	4.73	0.460
		40	13.99	57.42	40.02	6.74	0.470
		50	13.94	71.36	49.74	6.34	0.473
		60	13.69	85.05	59.28	6.59	0.487
		70	13.71	98.76	68.84	6.38	0.501
		80	14.12	112.88	78.69	5.37	0.512
		90	14.69	127.57	88.93	3.40	0.520
		100	15.88	143.44	100.00	.....	.....
		Average	.....	.....	.....	.....	0.4758

TABLE 15 (*continued*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M - R$	l
Second	A	10	12.52	12.52	9.59	2.62	0.597
		20	12.31	24.83	19.00	4.80	0.622
		30	12.20	37.03	28.34	6.67	0.645
		40	12.25	49.28	37.72	8.04	0.656
		50	12.36	61.64	47.18	8.90	0.662
		60	12.52	74.16	56.76	9.11	0.672
		70	12.84	87.00	66.58	8.64	0.680
		80	13.37	100.37	76.81	7.25	0.691
		90	14.22	114.59	87.70	4.63	0.710
		100	16.07	130.66	100.00	.....	.....
		Average	.....	.....	.....	.....	0.6594
	B	10	16.62	16.62	10.02	2.18	0.498
		20	16.52	33.14	19.97	3.83	0.498
		30	16.22	49.36	29.74	5.27	0.510
		40	16.12	65.48	39.46	6.30	0.515
		50	16.07	81.55	49.14	6.94	0.517
		60	16.02	97.57	58.79	7.08	0.522
		70	16.07	113.64	68.47	6.75	0.531
		80	16.47	130.11	78.40	5.66	0.541
		90	17.17	147.28	88.74	3.59	0.549
		100	18.67	165.95	100.00	.....	.....
		Average	.....	.....	.....	.....	0.5201
	C	10	13.51	13.51	9.88	2.32	0.530
		20	13.46	26.97	19.71	4.09	0.530
		30	13.31	40.28	29.44	5.57	0.538
		40	13.11	53.39	39.03	6.73	0.550
		50	13.16	66.55	48.65	7.43	0.553
		60	13.11	79.66	58.23	7.64	0.564
		70	13.31	92.97	67.96	7.26	0.570
		80	13.71	106.68	77.98	6.08	0.580
		90	14.31	120.99	88.44	3.89	0.595
		100	15.81	136.80	100.00	.....	.....
		Average	.....	.....	.....	.....	0.5567

TABLE 15 (*continued*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M - R$	l
Third	A	10	9.66	9.66	9.60	2.60	0.595
		20	9.56	19.22	19.11	4.69	0.605
		30	9.46	28.68	28.51	6.50	0.628
		40	9.46	38.14	37.91	7.85	0.640
		50	9.56	47.70	47.42	8.66	0.643
		60	9.61	57.31	56.97	8.90	0.655
		70	9.81	67.12	66.72	8.50	0.669
		80	10.26	77.38	76.92	7.14	0.680
		90	10.96	88.34	87.81	4.52	0.686
		100	12.26	100.00	100.00	.....	.....
		Average	.....	.....	.....	.....	0.6446
	B	10	8.16	8.16	9.69	2.51	0.574
		20	7.99	16.15	19.18	4.62	0.600
		30	8.03	24.18	28.71	6.30	0.610
		40	8.03	32.21	38.25	7.51	0.613
		50	7.99	40.20	47.74	8.34	0.621
		60	8.16	48.36	57.43	8.44	0.623
		70	8.20	56.56	67.17	8.05	0.633
		80	8.51	65.07	77.27	6.79	0.648
		90	9.07	74.14	88.04	4.29	0.656
		100	10.07	84.21	100.00	.....	.....
		Average	.....	.....	.....	.....	0.6198
	C	10	19.07	19.07	10.40	1.80	0.410
		20	18.82	37.87	20.67	3.13	0.407
		30	18.52	56.41	30.77	4.24	0.412
		40	17.97	74.38	40.57	5.19	0.424
		50	17.72	92.10	50.23	5.85	0.436
		60	17.52	109.62	59.78	6.09	0.450
		70	17.57	127.19	69.37	5.85	0.460
		80	17.77	144.96	79.06	5.00	0.480
		90	18.42	163.38	89.10	3.23	0.494
		100	19.97	183.35	100.00	.....	.....
		Average	.....	.....	.....	.....	0.4414

TABLE 15 (*concluded*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M-R$	l
Fourth	A	10	12.61	12.61	9.43	2.77	0.631
		20	12.41	25.02	18.72	5.08	0.658
		30	12.41	37.43	28.00	7.01	0.678
		40	12.46	49.89	37.33	8.43	0.686
		50	12.51	62.40	46.69	9.39	0.697
		60	12.71	75.11	56.19	9.68	0.712
		70	13.16	88.27	66.04	9.18	0.722
		80	13.71	101.98	76.29	7.77	0.741
		90	14.76	116.74	87.34	4.99	0.764
		100	16.92	133.66	100.00	.....	.....
		Average	.....	.....	.....	.....	0.6988
	B	10	13.58	13.58	10.17	2.03	0.465
		20	13.37	26.95	20.18	3.62	0.472
		30	13.21	40.16	30.08	4.93	0.477
		40	13.00	53.16	39.81	5.95	0.486
		50	12.89	66.05	49.46	6.62	0.495
		60	12.84	78.89	59.08	6.79	0.501
		70	12.94	91.83	68.78	6.44	0.506
		80	13.26	105.09	78.70	5.36	0.502
		90	13.63	118.72	88.91	3.42	0.522
		100	14.80	133.52	100.00	.....	.....
		Average	.....	.....	.....	.....	0.4918
	C	10	17.46	17.46	10.22	1.98	0.451
		20	17.22	34.68	20.30	3.50	0.454
		30	17.10	51.78	30.31	4.70	0.456
		40	16.90	68.68	40.20	5.56	0.456
		50	16.70	85.38	49.98	6.10	0.456
		60	16.62	102.00	59.70	6.17	0.455
		70	16.58	118.58	69.35	5.87	0.462
		80	16.70	135.28	79.18	4.88	0.464
		90	17.18	152.46	89.24	3.09	0.471
		100	18.38	170.84	100.00	.....	.....
		Average	.....	.....	.....	.....	0.4583

TABLE 16. RESULTS OF THE DUCLAUX DISTILLATION OF ACIDS OBTAINED BY OXIDATION OF THE ALCOHOLS OF FECES OF YOUNG CALVES

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M - R$	l
First	A	10	7.59	7.59	8.07	4.13	0.944
		20	7.84	15.43	16.41	7.39	0.954
		30	8.03	23.46	24.96	10.05	0.967
		40	8.28	31.74	33.77	11.99	0.972
		50	8.53	40.27	42.83	13.25	0.981
		60	9.06	49.33	52.47	13.40	0.992
		70	9.55	58.88	62.64	12.58	0.986
		80	10.09	68.97	73.37	10.68	1.018
		90	11.56	80.53	85.67	6.67	1.022
		100	13.47	94.00	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9818
	B	10	1.82	1.82	7.80	4.40	1.005
		20	1.96	3.78	16.21	7.59	0.980
		30	1.98	5.76	24.70	10.31	0.991
		40	2.58	7.84	33.60	12.16	0.986
		50	2.14	9.98	42.77	13.31	0.985
		60	2.26	12.23	52.44	13.43	0.987
		70	2.37	14.60	62.61	12.61	0.988
		80	2.55	17.15	73.54	10.51	1.004
		90	2.85	19.95	85.74	6.60	1.012
		100	3.33	23.32	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9931
	C	10	2.35	2.35	8.13	4.07	0.930
		20	2.39	4.74	18.41	7.39	0.954
		30	2.42	7.15	24.78	10.23	0.983
		40	2.53	9.68	33.55	12.21	0.991
		50	2.67	12.35	42.78	13.30	0.987
		60	2.80	15.15	52.49	13.38	0.982
		70	2.94	18.09	62.68	12.54	0.983
		80	3.21	21.30	73.80	10.25	0.976
		90	3.53	24.83	86.03	6.31	0.968
		100	4.03	28.86	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9727

TABLE 16 (*continued*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M - R$	l
		10	12.66	12.66	7.88	4.32	0.987
		20	13.21	25.87	16.09	7.71	0.997
		30	13.71	39.58	24.62	10.39	0.999
		40	14.26	53.84	33.49	12.27	0.995
		50	14.76	68.60	42.67	13.41	0.994
	A	60	15.47	84.07	52.30	13.57	1.000
		70	16.42	100.49	62.51	12.71	0.997
		80	17.67	118.16	73.50	10.55	1.007
		90	19.67	137.83	85.74	6.60	1.010
		100	22.92	160.75	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9984
		10	10.36	10.36	7.92	4.28	0.980
		20	10.81	21.17	16.18	7.62	0.973
		30	11.26	32.43	24.79	10.22	0.982
		40	11.56	43.99	33.63	12.13	0.983
		50	12.26	56.25	43.00	13.08	0.968
Second	B	60	12.61	68.86	52.64	13.23	0.972
		70	13.36	82.22	62.85	12.37	0.970
		80	14.41	96.83	73.86	10.19	0.970
		90	15.87	112.50	85.99	6.30	0.972
		100	18.32	130.82	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9744
		10	9.81	9.81	8.08	4.12	0.942
		20	10.13	19.94	16.41	7.39	0.955
		30	10.45	30.39	25.02	9.99	0.961
		40	10.69	41.08	33.82	11.94	0.968
		50	11.09	52.17	42.96	13.12	0.971
	C	60	11.61	63.78	52.52	13.35	0.980
		70	12.25	76.03	62.60	12.62	0.989
		80	13.34	89.37	73.59	10.46	1.000
		90	14.70	104.07	85.68	6.66	1.020
		100	17.38	121.45	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9762

TABLE 16 (*continued*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M-R$	l
		10	7.65	7.65	8.42	3.78	0.862
		20	7.81	15.46	17.02	6.78	0.876
		30	7.89	23.35	25.71	9.30	0.896
		40	8.09	31.44	34.61	11.15	0.905
		50	8.29	39.73	43.74	12.34	0.913
	A	60	8.61	48.34	53.22	12.65	0.931
		70	9.05	57.39	63.18	12.04	0.944
		80	9.73	67.12	73.90	10.15	0.967
		90	10.85	77.97	85.84	8.50	1.000
		100	12.86	90.83	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9216
		10	13.10	13.10	7.86	4.34	0.991
		20	13.74	26.84	16.11	7.69	0.992
		30	14.22	41.06	24.65	10.36	0.996
		40	14.70	55.76	33.47	12.29	0.997
		50	15.30	71.06	42.66	13.42	0.993
Third	B	60	15.94	87.00	52.23	13.64	1.005
		70	16.86	103.86	62.34	12.88	1.009
		80	18.30	122.16	73.33	10.72	1.023
		90	20.31	142.47	85.52	6.82	1.047
		100	24.11	166.58	100.00	.....	.....
		Average	.....	.....	.....	.....	1.006
		10	13.68	13.68	8.16	4.04	0.924
		20	13.87	27.55	16.44	7.36	0.950
		30	14.20	41.75	24.91	10.10	0.972
		40	14.71	56.46	33.69	12.07	0.978
		50	15.04	71.50	42.66	13.42	0.994
	C	60	16.25	87.75	52.35	13.52	0.994
		70	17.33	105.08	62.69	12.53	0.982
		80	18.91	123.99	73.97	10.08	0.960
		90	20.97	144.96	86.48	5.86	0.900
		100	22.65	167.61	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9616

TABLE 16 (*concluded*)

Trial	Calf	Volume of distillate (cubic centimeters)	N/10 acid (cubic centimeters)	Total amount of acid (cubic centimeters)	Per cent of acid, or R	$M-R$	l
Fourth	A	10	9.95	9.95	8.61	3.59	0.818
		20	10.09	20.04	17.34	6.46	0.835
		30	10.23	30.27	26.20	8.81	0.850
		40	10.27	40.54	35.09	10.67	0.866
		50	10.51	51.05	44.18	11.90	0.855
		60	11.07	62.12	53.76	12.11	0.881
		70	11.68	73.80	63.87	11.35	0.890
		80	12.51	86.31	74.70	9.35	0.890
		90	13.73	100.04	86.58	5.76	0.882
		100	15.50	115.54	100.00	.....	.....
		Average	.....	.....	.....	.....	0.8632
	B	10	7.53	7.53	8.22	3.98	0.908
		20	7.42	14.75	16.55	7.25	0.936
		30	7.57	22.32	25.04	9.97	0.958
		40	7.80	30.12	33.79	11.97	0.971
		50	8.03	38.15	42.80	13.28	0.983
		60	8.41	46.56	52.23	13.64	1.005
		70	8.92	55.48	62.24	12.98	1.016
		80	9.71	65.19	73.13	10.92	1.041
		90	10.83	76.02	85.28	7.06	1.085
		100	13.12	89.14	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9892
	C	10	14.90	14.90	7.88	4.32	0.987
		20	15.55	30.45	16.11	7.69	0.992
		30	16.06	46.51	24.60	10.41	1.001
		40	16.77	63.28	33.47	12.29	0.998
		50	17.33	80.61	42.64	13.44	0.995
		60	18.31	98.92	52.32	13.55	0.996
		70	19.29	118.21	62.52	12.70	0.995
		80	20.78	138.99	73.52	10.53	1.005
		90	22.93	161.92	85.65	6.69	1.026
		100	27.13	189.05	100.00	.....	.....
		Average	.....	.....	.....	.....	0.9994

Memoir 78, *A Study of the Leaf-mining Diptera of North America*, the twelfth preceding number in this series of publications, was mailed on April 14, 1925.

Memoir 79, *A Biological Study of Aphelinus mali Hald., a Parasite of the Woolly Apple Aphid, Eriosoma lanigera Hausm.*, was mailed on October 9, 1924.